Turkish Journal of Fisheries and Aquatic Sciences 8: 37-41 (2008)

Microcystis aeruginosa Bloom and the Occurrence of Microcystins (Heptapeptides Hepatotoxins) From an Aquaculture Pond in Gazipur, Bangladesh

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Received 24 September 2007
Accepted 02 January 2008

Abstract

Bangladesh is a tropical country of large alluvial plain with 1.3 million freshwater ponds and lakes and has a proper environment for luxuriant growth of cyanobacteria. Algal bloom of *Microcystis aeruginosa* occurred in an aquaculture pond in Gazipur, Dhaka. Bloom sample was collected and filtered through a glass fiber filter. Methanol-water extract of filtered cells was analyzed by high performance liquid chromatography (HPLC) with UV, MS and MS-MS detection, detected three types of microcystins *viz.*, Microcystin-RR, Microcystin-YR and Microcystin-LR and those were confirmed by HPLC-MS. The amount of MC-LR was the highest (33.2 μg L^{-1}) followed by MC-RR (9.03 μg L^{-1}) and MC-YR (5.23 μg L^{-1}). The concentration of microcystins was well above the WHO provisional guideline value of 1 μg L^{-1} MC-LR. Further investigations need to characterize other types of microcystins from bloom forming cyanobacteria and their effect on human health and cultured fish in Bangladesh.

Key words: Microcystis aeruginosa, microcystin, HPLC, algal bloom, Bangladesh.

Introduction

There are over 30 species of cyanobactria that can be associated with toxic water blooms (Skulberg *et al.*, 1993) and reports are available from at least 44 countries and from the Baltic and Caribbean Seas, and Atlantic, Pacific and Indian Oceans (Carmichael, 1989; Codd, 1995). Eutrophication of freshwaters and appearance of cyanobacterial bloom, have become a worldwide problem which can become serious when bloom-forming species release potent water soluble toxins (Watanabe and Oishi, 1980; Vasconcelos *et al.*, 1993; Carmichael, 1994). Toxic cyanobateria are now recognized as a hazard to human and animal welfare and health assessments are being carried out to determine environmental heath problems (Skulberg *et al*., 1984; Carmichael, 1994; 1995). Bangladesh is a densely populated country with 138 million people living in a land mass of only 147.5 thousand km^2. Fish is the major sourc of animal of protein (80%) for its overgrowing population. Recently, aquaculture has spread quite rapidly and became the major source of fish accounting for 43% of the total fish production of the country compared to 1% in 1970s (Karim *et al.*, 2006). Traditionally, aquaculture in Bangladesh has been realized in the form of extensive pond culture of freshwater species mainly major craps and catfish. The current trend is towards more intensive methods with high stocking densities and excessive supplementary feed leading to eutrophication of ponds.

There are about 1.3 million fresh and brackish water ponds (FRSS, 1986), which account for only 3.5 percent of the inland waters of Bangladesh but contribute about 31% of inland fish production. In Bangladesh, most fish ponds are rain-fed and have multiple uses such as washing clothes, household, and kitchen items; serving as crop irrigation and drinking water for livestock; and even being used for bathing. Cyanobacteria (*Microcystis)* blooms are frequently occurred in these ponds and lakes (Islam and Nahar, 1967; Islam and Uddin, 1977; Aziz, 1974; Islam, 1991). However, these blooms have been poorly studied. This paper deals with isolation and characterization of microcystins from a natural bloom of *M. aeruginosa* occurring in an aquaculture pond in Gazipur, Dhaka.

Materials and Methods

The study pond is located in Gazipur district (90°21′ E longitude 24°00′ N latitude) 20 km north from Dhaka city (Figure 1). The pond is 0.1 ha in size and stocked with catfish, *Pangasius pangasius*. Algal bloom (*M. aeruginosa*) was initiated in the first week of March 2005 and the highest cell density (95% *Microcystis*) was recorded on March 10, 2005. The bloom sample was collected with plankton net of 20 μm mesh size. A portion of (5 ml) of the concentrated samples were filtered through an 0.45 μm glass fiber filter (Whatman GF/C, 47 mm diameter) and dried in an oven at 60-80°C. Dried filters covered with algae cells were transported to the Alfred Wegner Institute, Sylt, Germany for analysis.

Extraction

GF/C filters and 1.0 ml of a mixture of water

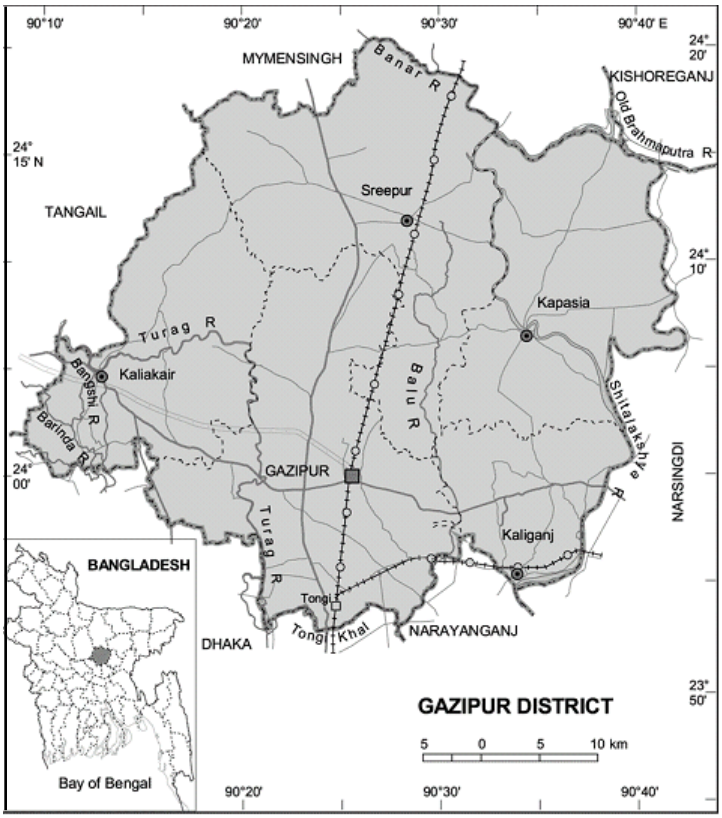

Figure 1. Map of the study area, Gazipur.

and methanol (50:50; v:v) was sonicated for 20 minutes and centrifuged (3,000 g). The supernatant was filtered through a nylon filter with 0.45 µm pore size.

Chemical Analysis

The HPLC/UV determination of microcystins was carried out following the methods of Lawton *et al.* (1994) with some modifications (Hummert *et al*., 2001a; C18 column: Phenomenex prodigy, ODS (3), 250 x 4.6 mm, 5 µm, mobil phases: acetonitrile /water/0.05% TFA). Detection of microcystins was done by the use of an UV detector (Shimadzu SPD-10AV; λ=238 nm). HPLC/MS and HPLC/MS-MS analysis were applied to ensure the identity of the toxin peaks in the chromatograms. The HPLC was coupled by means of an electrospray interface to a single quadrupol mass spectrometer (API 150, PE Sciex Instruments, Canada) and additional to a triple quadrupol mass spectrometer (API 365, PE Sciex Instruments, Canada). The detection was carried out in selected ion monitoring (SIM) mode using LC/MS and multiple reactions monitoring mode (MRM) using LC/MS-MS (Hummert *et al.*, 2001b).

Microcystins and Nodularin Standards

Standards of Microcystin-RR, Microcystin-LR, Microcystin-YR, Microcystin-LA and Nodularin were purchased from Calbiochem/Novabiochem (La Jolla, CA, USA).

Chemicals

HPLC grade acetonitrile and HPLC grade methanol were purchased from Baker (Deventer, Netherlands). Water was purified to HPLC grade quality with a Millipore-Q RG Ultra Pure Water System (Millipore, Milford, USA).

Results and Discussion

In the original bloom sample the cell density of *M. aeruginosa* was 6.22 x 10^8 cells L^{-1} . During the bloom dissolved oxygen, free carbon dioxide and nitrite nitrogen of pond water were recorded as 4.5, 16.0 and 0.68 mg L^{-1} respectively. The pH was 8.5 and the water temperature was between 20-24ºC. HPLC analysis of *M. aeruginosa* extract showed three peaks, the retention time of which agreed well with standard MC-RR, MC-YR and MC-LR (Figure 2). The results of HPLC-MS revealed the identification of three variants of microcystins (Figure 3), according to their corresponding molecular weight: MC-LR (at m/z 995.0 $[M+H]^+$), MC-RR (at m/z 519.5 $[M+2H]^{2+}$) and MC-YR (at m/z 1045.0 $[M+H]^+$). In *M. aeruginosa* sample the amount of MC-LR was the

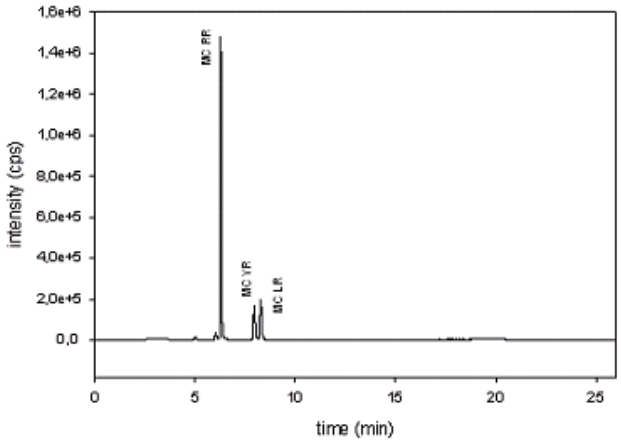

Figure 2. HPLC-MS chromatograms of *Microcystis aerguinosa* (filtered cells) collected from Gajipur, Dhaka.

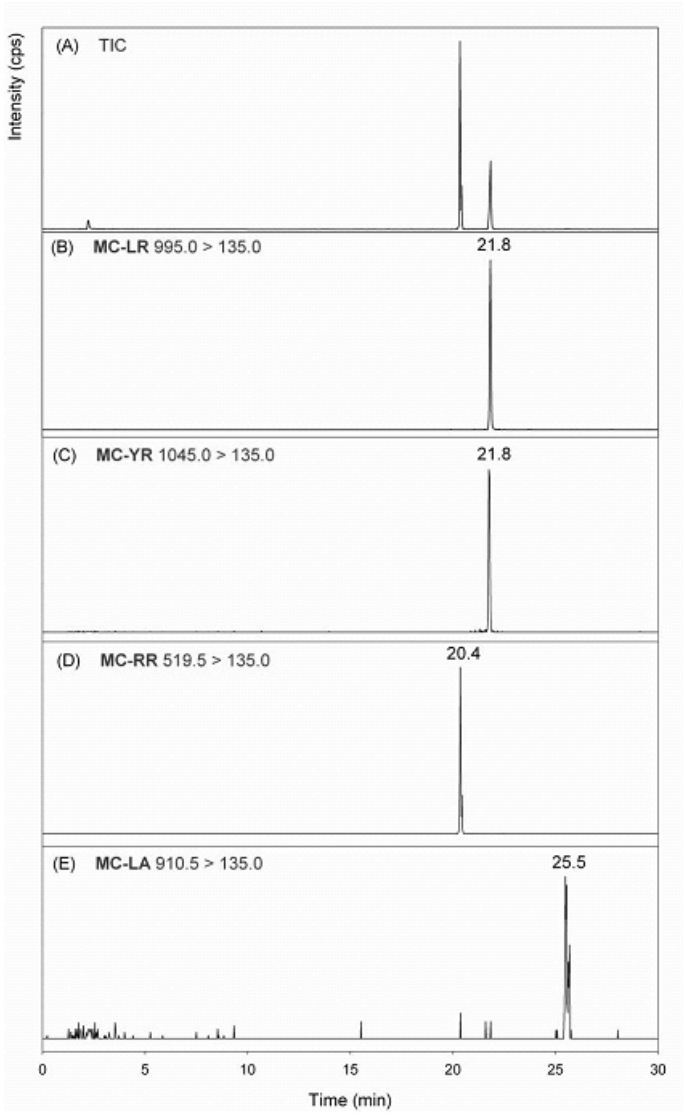

Figure 3. HPLC/MS-MS chromatogram of microcystins detected from *Microcystis aerguinosa* (filtered cells).
(A) TIC; (B) Microcystin-LR, $[MC\text{-}LR+H]^+$ 995.0 > 135.0; (C) Microcystin-YR, $[MC\text{-}YR+H]^+$ 1045.0 > 135.0;
(D) Microcystin-RR $[MC\text{-}RR+2H]^{2+}$ 519.5 > 135.0; (E) Microcystin-LA $[MC\text{-}LA+H]^+$ 910.5 > 135.0.

highest (33.2 μg L^{-1}) followed by MC-RR (9.03 μg L^{-1}) and MC-YR (5.23 μg L^{-1}). A small amount of MC-LA was also detected. Welker *et al.* (2004), in a study at three different regions in Bangladesh detected microcystins in 39 ponds, mostly together with varying abundance of potentially microcystin-producing genera such as *Microcystis*, *Planktothrix* and *Anabaena*. Total microcystin concentrations in their study ranged between <0.1 and up to >1000 μg L^{-1}, and more than half of the positive samples contained high concentrations of more than 10 μg L^{-1}. Our results clearly showed that the concentration of microcystins is well above the WHO provisional guideline value of 1 μg L^{-1} MC-LR. In Australia, a safety factor for tumor promotion is 1.0 μg microcystins or nodularins L^{-1} (Falconer *et al.*, 1994). In Canadian drinking water, maximum accepted concentration for MC-LR is 0.5 mg L^{-1} and for other microcystins, 1 μg L^{-1} of total microcystins (Carmichael, 1995).

The occurrence of *M. aeruginosa* blooms in lake/pond that produce hepatotoxic microcystins is a problem, especially if the water is utilized as drinking supply and/or for recreational purposes. Epidemiological investigations have demonstrated that microcystins cause stomach and intestinal inflammation, liver cancer and disease of the spleen in humans who drink water containing microcystins (McDermott *et al.*, 1998; Ding *et al.*, 2000; Zhou *et al.*, 2002). In Bangladesh, local people use pond/lakes water for aquaculture or domestic uses even when bloom or scum is formed as they have no knowledge about toxicity and in some cases they have no alternative.

Although there is no official record of animal or human intoxication induced by cyanobacteria, the effect of microcystins on aquatic animals and human through direct exposure or food chain remains to be identified.

Acknowledgements

The financial support from Intergovernmental Oceanographic Commission (IOC) of UNESCO to Dr. Md. Sagir Ahmed, travel to Germany to analyze the sample is greatly acknowledged. We thank two anonymous reviewers for their valuable comments on the earlier version of the present paper.

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